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Mutagenic Analysis of the F_0 Stator Subunits

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The *a* and *b* subunits constitute the stator elements in the F_0 sector of F_1F_0-ATP synthase. Both subunits have been difficult to study by physical means, so most of the information on structure and function relationships in the *a* and *b* subunits has been obtained using mutagenesis in combination with biochemical methods. These approaches were used to demonstrate that the *a* subunit in association with the ring of *c* subunits houses the proton channel through F_1F_0-ATP synthase. The map of the amino acids contributing to the proton channel is probably complete. The two *b* subunits dimerize, forming an extended flexible unit in the peripheral stalk linking the F_1 and F_0 sectors. The unique characteristics of specific amino acid substitutions affecting the *a* and *b* subunits suggested differential effects on rotation during F_1F_0-ATPase activity.

KEY WORDS: F_1F_0 ATP synthase; stator; F_0.

INTRODUCTION

Mitochondrial, chloroplast, and bacterial F_1F_0-ATP synthases use the energy of the electrochemical gradient of protons across membranes to drive ATP synthesis. In facultative bacteria, growing under anaerobic conditions, F_1F_0-ATP synthase functions as an ATP-driven proton pump, maintaining the membrane gradient required for other membrane-associated activities. Regardless of the biological source, all F_1F_0-ATP synthases have both the ATP synthesis and the ATP hydrolysis activities *in vitro*. The functional similarities between F_1F_0-ATP synthases are reflected in their common molecular architecture (Fig. 1).

F_1 sectors consist minimally of the $\alpha_3\beta_3\gamma\delta\epsilon$ subunits (*Escherichia coli* nomenclature). High-resolution structures have been obtained for the bovine and rat $\alpha_3\beta_3\gamma$ subunits (Abrahams *et al.*, 1994; Bianchet *et al.*, 1998). The inherent asymmetry observed in the bovine structure suggested that the differing binding affinities observed in Boyer's pioneering kinetic studies resulted from differential interactions between each of the three $\alpha\beta$ subunit pairs and the γ subunit (Boyer, 1997; Nakamoto *et al.*, 1999). A series of elegant experiments conducted on bacterial F_1 demonstrated that the γ and ϵ subunits rotated relative to the $\alpha_3\beta_3$ hexamer cycling the three catalytic sites through each of three distinct conformational states (Duncan *et al.*, 1995; Sabbert *et al.*, 1997; Noji *et al.*, 1997; Kato-Yamada *et al.*, 1998; Bulygin *et al.*, 1998).

Bacterial F_0 sectors contain the intrinsic membrane subunits ab_2c_{10-12}. Nuclear magnetic resonance studies have shown that *c* spans the membrane twice, forming a hairpin loop on the F_1 side of the membrane (Girvin *et al.*, 1998). The F_0 *c* subunits are arranged in a ring (Rastogi and Girvin, 1999; Fillingame *et al.*, 2000) and the driving force for rotation of the γ and ϵ subunits appears to be rotation of this *c*-subunit oligomer (Sambongi *et al.*, 1999). The number of *c* subunits in F_1F_0-ATP synthase remains controversial. Clearly there can be as many as twelve *c* subunits in F_1F_0-ATP synthase complexes (Jones and Fillingame, 1998). Twelve is an accommodating number from the mechanistic viewpoint of having a multiple of three *c* subunits

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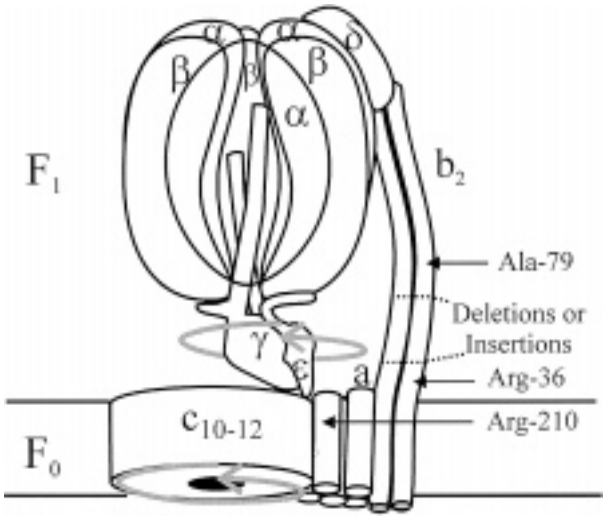

Fig. 1. Gross structure of the *E. coli* F_1F_0-ATP synthase. The approximate positions of the R210 of the *a* subunit and R36 and A79 of the *b* subunit are marked. Deletion and insertion mutations were constructed in the area of the *b* subunits shown corresponding to the segment visible in electron micrographs as the peripheral stalk between the F_1 and F_0 sectors.

for the three-step rotation in F_1. However, variation in the number of *c* subunits has been observed (Schemidt *et al.*, 1998) and the recent low-resolution structure of the yeast enzyme had only ten *c* subunits (Stock *et al.*, 1999).

Electron microscopy of the *E. coli* enzyme showed that F_1 was linked to F_0 by two narrow stalk structures about 40 Å in length (Wilkens and Capaldi, 1998). The central stalk was the $\gamma\epsilon$ rotor and the peripheral stalk consisted of the hydrophilic portions of the two *b* subunits. The *b* subunits were in contact with the α, β, and δ subunits in F_1 (Rodgers and Capaldi, 1998; McLachlin *et al.*, 2000). The current model proposes that the peripheral stalk holds the $\alpha_3\beta_3$ hexamer in place against the rotation of the central stalk. This functional distinction gave rise to the concept of the stator subunits defined as those that do not rotate relative to the $\alpha_3\beta_3$ hexamer.

At the present time, the only subunits that have not yet been successfully studied by high-resolution structural analysis are the F_0 stator subunits. As a consequence, much of what is known about structure and function relationships for the *a* and *b* subunits has been determined by application of a combination of recombinant DNA technology and traditional biochemistry methods. The work on the *a* and *b* subunits is the focus of the present review.

THE *a* SUBUNIT

The *a* subunit is the largest protein in F_0 and is present in a stoichiometry of one per enzyme complex. Like most integral membrane proteins, it is extremely hydrophobic. The Fillingame and Vik laboratories independently arrived at the conclusion that the *a* subunit spanned the membrane five times (Fillingame *et al.*, 2000; Wada *et al.*, 1999). Both groups agreed that the amino terminus was exposed to the periplasmic space and the carboxyl terminus resided in the cytoplasm. At least some of the earlier topology work can be rationalized within the framework provided by these two studies, so it seems reasonable to accept the five membrane-span topology for the *a* subunit.

All F_1F_0-ATP synthases contain a protein with primary sequence homology to the *a* subunit (Hartzog, 1994). In mitochondria this subunit is referred to as the ATPase-6 subunit. Primary sequences of *a* subunits display a striking degree of primary sequence homology in the apparent fourth and fifth membrane-spanning segments and several specific amino acids are invariant. The presence of only one *a* subunit demands that F_0 be an inherently asymmetric structure. Electron microscopy of isolated F_0 confirmed this and the micrographs implied that the *a* subunit is located to one side of the ring of *c* subunits (Birkenhäger *et al.*, 1995; Singh *et al.*, 1996).

a Subunit in Proton Translocation

Cain and Simoni (1986) presented the first tangible evidence that the *a* subunit might be a direct participant in F_0-mediated proton translocation. An extensive mutant search was conducted on the *uncB(a)* gene using failure to grow on succinate medium as a screen for defects in F_1F_0-ATP synthase. Among the collection of mutants were the first two single amino acid replacements characterized in the *a* subunit, S206L and H245Y. The H245Y substitution was more interesting because assembly of the F_1F_0 complex appeared to be normal, but F_1F_0-ATP synthase function was undetectable. H245Y was located at a strongly conserved position in the *a* subunit and S206 was adjacent to a conserved site, so it seemed plausible that the *a* subunit housed at least part of the F_0 proton channel. We and several other laboratories set out to map the amino acids contributing to proton translocation. More than one-hundred mutations were constructed by site-directed mutagenesis explicitly for this purpose and

others have been characterized in the course of more sophisticated experiments. All of the obviously conserved amino acids have been investigated by construction of multiple replacements. In general, the mutations in the conserved positions impaired F_0-mediated proton translocation, but the severity of the defects varied widely. Present models propose that the *a* subunit contributes two half channels providing access to the site of protonation, *c* subunit D61, from the periplasmic space and the cytoplasm, respectively.

R210

The only amino acid in the *a* subunit absolutely essential for F_1F_0-ATP synthase function was R210. Mutations altering R210 invariably abolished F_1F_0-ATP synthase activity. Substitution with basic, acidic or bulky nonpolar amino acids blocked proton movement through F_0 (Lightowlers *et al.*, 1987; Cain and Simoni, 1989; Eya *et al.*, 1991). Both ATP-driven proton pumping and passive F_0-mediated ion conduction were affected. These effects were not the result of a failure to assemble the F_1F_0 complex. Releasing F_1 from membrane vesicles prepared from mutant strains revealed abundant ATPase activity suggesting the presence of stable F_1F_0 complexes containing the altered *a* subunits. This interpretation has been directly confirmed by purifying intact F_1F_0-ATPase containing the R210I *a* subunit using standard procedures for the F_1F_0-ATP synthase (Gardner and Cain, 1999).

A recurring observation for mutations affecting F_0 was that if enough missense mutations affecting a single site were studied, then some were likely to display differing biochemical properties. In this case, the R210A substitution provided unusual results (Valiyaveetil and Fillingame, 1997). The R210A substitution left the F_0 proton channel intact as judged by passive conduction. Therefore, the mutation had the rarest of phenotypes in which both F_1 and F_0 are functional but were not obligately coupled. Another area of consternation arose from the observation that amino acid substitutions, which inhibited proton translocation, often reduced F_1F_0-ATP hydrolysis activity and displayed reduced sensitivity to dicyclohexylcarbodiimide (DCCD). However, others like the R210I replacement did not strongly inhibit F_1F_0-ATPase activity and retained DCCD inhibition (Gardner and Cain, 1999). The position of the central stalk ϵ subunit was known to differ depending on whether rotation was occurring as a result of F_1F_0-ATP hydrolysis or the enzyme was substrate inhibited (Mendel-Hartvig and Capaldi, 1991). Results very similar to the wild-type enzyme were observed for purified R210I F_1F_0-ATPase in proteolysis and chemical cross-linking experiments (Gardner and Cain, 1999). Thus, R210I provided an example of a single amino acid substitution uncoupling active F_1-ATPase capable of rotation from a defective F_0 within an intact F_1F_0 complex.

What is the role of R210 in proton translocation? Since R210 was absolutely essential for F_1F_0-ATP synthase function, arguments have been made that it may undergo direct protonation–deprotonation. Arginine seems like an unlikely candidate for such a role. In the current topological models, R210 was positioned in the fourth membrane span and the site was deeply buried within the membrane bilayer at a level close to the only other mechanistically essential amino acid in F_0, D61 of the *c* subunit (Fillingame *et al.*, 2000). Therefore, the suggestion that R210 modulates protonation of D61 in the *c* subunits is an interesting possibility (Rastogi and Girvin, 1999).

G218, E219, and H245

Aside from R210, the two most intensely studied sites in the *a* subunit are E219 and H245 located in the fourth and fifth membrane spans, respectively. Mutations at either site had a significant impact on F_0 proton translocation (Cain and Simoni, 1989; Hartzog and Cain, 1994; Hatch *et al.*, 1998). As a result, much has been written about whether these amino acids were necessary. The answer was emphatically *no*. Neither E219, nor H245 were essential for proton translocation because replacement of either with several different amino acids allowed detectable levels of F_1F_0-ATP synthase activity. Nevertheless, both sites were demonstrated to share an important role influencing F_0 proton conductance.

The positions occupied by G218, E219, and H245Y in the *a* subunit may represent an example of coevolution, since specific arrangements of amino acids in these sites can be associated with specific branches of heredity. The theory states that a mutation affecting a functionally important site within a protein would likely be accompanied by a second compensating mutation causing the two positions to pass through evolution as a unit, rather than as independent sites with unrelated rates of mutation. Mitochondria, chloroplasts, alkaliphilic bacteria, and other eubacteria *a* subunits each have distinctive constellations of amino

acids at the three positions. Double mutants have been constructed mimicking each of the other lines of evolution within the *E. coli a* subunit to investigate whether the three amino acids had a functional interaction (Cain and Simoni, 1988; Hartzog and Cain, 1994). In each case, the double mutations yielded substantially more activity than any of the corresponding single mutations.

While this site-directed suppressor approach established a functional relationship, it could not be interpreted with certainty in terms of a direct physical contact. However, in view of the fact that three different pairs of mutations were mutually suppressing, it seems most probable that the three sites were in immediate proximity to one another and involved in a direct interaction. Assuming that the fourth membrane span of the *a* subunit is an α helix, then E219 lies on the opposite face of the helix from R210. One possibility is that E219 and H245 function in the proton intake channel leading from the periplasm, rather than proton loading onto the *c* subunit involving R210. Another possibility is that the interactions between G218, E219, and H245 may be important in aligning the critical fourth membrane span.

Other Important Sites

The other strongly conserved amino acids along the fourth and fifth membrane-spanning segments of the *a* subunit also play important, but nonessential, roles (Cain and Simoni, 1989; Hartzog and Cain, 1993a; Hatch *et al.*, 1998; Kuo and Nakamoto, 2000). For example, N214 and Q252 were both universally conserved in *a* subunits of all F_1F_0-ATP synthases. Many mutants were constructed at each site, yielding a range of effects that might be expected for substitutions of amino acids lining a water-filled channel.

A217 was among the group of important, but nonessential, amino acids. A mutation at this site, A217R, has become the most thoroughly characterized single substitution in F_0. A217R represented a prototypical example of a substitution that both blocked the proton channel and inhibited F_1F_0-ATPase activity (Gardner and Cain, 1999). In this instance, the defect in F_0 was tightly coupled to catalysis. Preparation of intact F_1F_0 complexes containing the A217R subunit was straightforward. Examination of the ϵ subunit in the presence of Mg^{2+}-ATP suggested that it was in a state similar to that observed for substrate-inhibited form of F_1F_0-ATPase. Two different mechanisms might have accounted for these observations. Inhibition of ATP hydrolysis could have reflected a defect in rotation, or alternatively, the change in the ϵ subunit could have been propagated into the catalytic site. Since unisite catalysis was independent of rotation (Garcia and Capaldi, 1998), substantial differences in unisite catalysis would be indicative of structural perturbation of the catalytic sites rather than inhibition of rotation. Measurements of the unisite micro-rate constants for substrate binding and catalysis revealed insignificant differences between the A217R enzyme and authentic F_1F_0-ATP synthase (Gardner and Cain, 2000). Therefore, the F_1 catalytic sites seemed to be intact, suggesting that the most likely cause of inhibition of F_1F_0-ATPase by the A217R substitution was impairment of rotation. The differences in effects of amino acid substitutions on rotation within the F_1F_0-ATPase may be the source of variations between the biochemical phenotypes for mutations affecting F_0.

Modeling Human Disease Mutations

A growing number of mutations in the human ATPase-6 gene have been associated with specific cases of Leigh's Syndrome and NARP (Wallace, 1999). Loss of F_1F_0-ATP synthase was attributed to the mitochondrial DNA nt8993T → G (L156R), and later a second allele, nt8993T → C (L156P). ATPase-6 L156 occupies the site homologous to L207 of the *a* subunit in *E. coli*. Since there was strong conservation of this leucine, we reasoned that the biochemical defects resulting from the L207R and L207P replacements would be appropriate models for the biochemical defects in the human enzyme. Both *E. coli a* subunit L207 replacements resulted in decreases in F_1F_0-ATP synthase activity related to minor assembly defects and marked decreases in F_0-mediated proton translocation (Hartzog and Cain, 1993b, Hartzog *et al.*, 1999). The L207P substitution was less deleterious than L207R (Hartzog *et al.*, 1999), and this correlated well with the observation that patients with comparable levels of defective mitochondrial DNA had relatively milder symptoms with the nt8993T → C as compared to nt8993T → G patients (Rahman *et al.*, 1996). Ogilvie and Capaldi (1999) adopted a similar approach for modeling the ATPase-6 nt8993T → C disease mutation, as well as, several other disease related sites in the ATPase-6 gene. Decreased levels of F_1F_0-ATP synthase function were observed with the L207P substitution and the L262P change modeling the Leigh's Syndrome nt9815T → C mutation.

THE *b* SUBUNIT

The *E. coli* F_1F_0-ATP synthase has two identical *b* subunits (Fig. 1). Each spans the membrane once and has an extensive hydrophilic carboxyl terminal domain. Nuclear magnetic resonance spectroscopy suggested that the membrane-spanning segment formed an α helix that may be canted across the membrane (Dmitriev *et al.*, 1999). The hydrophilic portions of two *b* subunits formed a dimer of parallel α helices probably in a conformation similar to a coiled-coil (Revington *et al.*, 1999). This was the mass visualized as the peripheral stalk in F_1F_0-ATP synthase. Therefore, the *b* subunit is thought to provide the structural integrity linking stator elements of F_1 to F_0 holding the $\alpha_3\beta_3\delta$ subunits against rotation of the central stalk.

Mutagenesis of the *b* Subunit

Unlike the *a* subunit, the *b* subunit has very few obviously conserved amino acids to guide experiments. Secondary structure predictions suggested that the *b* subunit hydrophilic region had two long α helixes broken by a small area of random coil or possibly a β-turn. Since this was a potential structural feature dictating the overall shape of the subunit, targeted random mutagenesis was conducted on the *uncF(b)* gene to investigate the importance of the putative turn (McCormick and Cain, 1991). Only A79, predicted to be the final amino acid of the first α helix, was sensitive to substitution with amino acids other than proline. Altering A79 resulted in an assembly defect in F_1F_0-ATP synthase, stemming from an unstable *b* subunit (McCormick *et al.*, 1993). The predicted β turn was eventually set aside for two reasons. First, model peptides based on this segment of the *b* subunit showed no indication of turn formation (Sorgen *et al.*, 1998a), and, second, the *b* subunit was in an extended conformation reaching the δ subunit atop the F_1F_0 complex (Rodgers and Capaldi, 1998).

We hypothesized that the bases of the assembly defects induced by replacing A79 might be a failure to form the *b* subunit dimer. Dunn (1992) had successfully expressed the hydrophilic portion of the *b* subunit generating the b_{sol} polypeptide to model *b* subunit interactions. The b_{sol} protein formed dimers that specifically interacted with F_1. The collection of A79 mutants were transferred to the b_{sol} expression system to investigate the effects of the amino acid replacements on dimerization. Most of the b_{sol} proteins with changes at A79 retained α helical structures, but chemical crosslinking and sedimentation experiments suggested much less efficient dimerization (Sorgen *et al.*, 1998a). Moreover, the monomeric b_{sol} proteins apparently had little affinity for specific interactions with F_1. The level of competition for binding F_1 with authentic F_0 in a membrane was directly proportional to the concentration of dimers in the assays. Therefore, formation of the *b* subunit dimer is a likely early step in assembly of F_1F_0-ATP synthase.

Although it was unrecognized because of the absence of other conserved amino acids in the vicinity, A79 may be a conserved site among bacterial *b* subunits. With one exception, all known bacterial *b* subunits have an alanine at the end of the first predicted α helix. Exactly 43 amino acids in the amino terminal direction from A79, conserved R36 is positioned approximately two turns of the α helix above the surface of the membrane (Fig. 1). Missense mutations were constructed affecting R36 to study its function (Caviston *et al.*, 1998). The R36I and R36E mutants had virtually no growth on succinate medium indicating a loss of oxidative phosphorylation. Other R36 mutants had respectable levels of F_1F_0-ATP synthase function. The interesting result was that the R36I and R36E substitutions affected F_1F_0-ATP synthase by different mechanisms. F_1F_0 complexes formed with the R36I *b* subunit were severely limited in F_0 proton translocation, but the R36E substitution had readily detectable F_0 function. Indeed R36E had limited ATP proton-pumping capacity, suggesting a difference in the level of uncoupling F_1, depending on whether one measured ATP synthesis or ATP hydrolysis. Recently, McLachlin *et al.* (2000) positioned R36 in F_0 by showing crosslinking to the *a* subunit from the R36C mutant.

The Peripheral Stalk

The *b* dimer has been shown to interact with the α, β, and δ subunits (Rodgers and Capaldi, 1998; McLachlin *et al.*, 2000). The initial impression in the field was that the peripheral stalk was a rigid, rodlike structure. This was supported by the apparent conservation of secondary structures among bacterial *b* subunits and by the fixed distance between R36 and A79. Indeed, the mechanical demands of the rotary mechanism gave rise to the term "stator" for the nonrotating subunits implying fixed structural elements.

If the *b* subunit dimer was a rigid structure of fixed length, then only small changes in the length of

the peripheral stalk resulting from manipulation of the *b* subunit were likely to be accommodated by F_1F_0-ATP synthase. A series of deletions and insertions were constructed between R36 and A79 to test this hypothesis (Fig. 1). The important parameter in these experiments was to determine the largest insertions and deletions that could be constructed with the retention of function. The deletions were constructed first and it immediately became clear that unexpectedly large segments of the *b* subunit could be eliminated while retaining detectable enzyme function (Sorgen *et al.*, 1998b). Active F_1F_0-ATP synthase was found in three different deletions of 11 amino acids. Reduced amounts of activity was related to decreased levels of *b* subunit in the membranes, rather than a structural defect in the intact enzyme. Once formed, F_1F_0-ATP synthases with the shortened *b* subunits were functional. It was also possible to lengthen the *b* subunit by insertion of up to 14 amino acids and retain detectable ATP synthase activity (Sorgen *et al.*, 1999). Although the focus was on the maximum size of the alterations, it was notable that either insertion or deletion of seven amino acids yielded a very slight reduction in the number of assembled F_1F_0 complexes. These enzymes had biochemical characteristics identical to authentic F_1F_0-ATP synthase. Therefore, conservation of the length of the *b* subunit in the second stalk region did not reflect a mechanistic advantage for enzyme function, but likely reflected an evolutionary advantage favoring the most efficient size for F_1F_0 complex assembly.

These data demanded a tangible change in the model of the peripheral stalk. Assuming an ideal α helix, the deletions eliminated 16 Å and the insertions added 21 Å. These distances constituted about 40 to 50% of the measured distance between F_1 and F_0 (Wilkens and Capaldi, 1998). Apparently, there was slack in the peripheral stalk that could be either reduced or increased while continuing to satisfy the functional requirements of the *b* subunits. Rather than a stiff rod extending from F_0 to F_1, the peripheral stalk may be more of a rope or tether. According to this model, the tensile strength needed to hold F_1 against the rotation of the central stalk is provided by stretching the rope, rather than inherent rigidity in the *b* subunit coiled-coil.

CONCLUSIONS AND THE FUTURE

The combined genetic and biochemical approach has provided a wealth of information about the stator subunits in F_0. Probably all of the amino acids directly involved in forming the F_0 proton channel have been mapped. Only two amino acids proved absolutely essential for F_1F_0-ATP synthase activity, D61 in the *c* subunits and R210 in the *a* subunit. All others can be replaced by conservative amino acid substitutions yielding at least partially functional complexes. Substitutions, such as *a* subunit A217R, probably affect rotation during F_1F_0-ATPase activity. Inhibition of rotation may account for the differences in biochemical phenotypes seen in F_0 mutants. We no longer think of the *b* subunits as passive structural elements in a single rigid conformation, but consider the peripheral stalk to be a flexible structure bending and perhaps stretching with catalysis. Whether the apparent flexibility of the *b* subunit contributes to the elasticity and energy storage envisioned by Cherepanov *et al.* (1999) remains to be seen. The flexibility observed in the genetic experiments may explain the difficulties encountered in attempts to determine a high-resolution structure for b_{sol} and intact F_1F_0-ATP synthase.

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REFERENCES

Abrahams, J. P., Leslie, A. G. W., Lutter, R., and Walker, J. E. (1994). *Nature (London)* **370**, 621–628.
Bianchet, M. A., Hullihen, J., Pedersen, P. L., and Amzel, L. M. (1998). *Proc. Natl. Acad. Sci. USA* **95**, 11605–11070.
Birkenhäger, R., Hoppert, M., Deckers-Hebestreit, G., Mayer, F., and Altendorf, K. (1995). *Eur. J. Biochem.* **230**, 58–67.
Boyer, P. D. (1997). *Annu. Rev. Biochem.* **66**, 717–749.
Bulygin, V. V., Duncan, T. M., and Cross, R. L. (1998). *J. Biol. Chem.* **273**, 31765–31769.
Cain, B. D. and Simoni, R. D. (1986). *J. Biol. Chem.* **261**, 10043–10050.
Cain, B. D. and Simoni, R. D. (1988). *J. Biol. Chem.* **263**, 6606–6012.
Cain, B. D. and Simoni, R. D. (1989). *J. Biol. Chem.* **264**, 3292–3300.
Caviston, T. L., Ketchum, C. J., Sorgen, P. L., Nakamoto, R. K., and Cain, B. D. (1998). *FEBS Lett.* **429**, 201–206.
Cherepanov, D. A., Mulkidjanian, A. Y., and Junge, W. (1999). *FEBS Lett.* **449**, 1–6.

Dmitriev, O., Jones, P. C., Jiang, W., and Fillingame, R. H. (1999). *J. Biol. Chem.* **274**, 15598–15604.
Duncan, T. M., Bulygin, V. V., Zhou, Y., Hutcheon, M. L., and Cross, R. L. (1995). *Proc. Natl. Acad. Sci. USA* **92**, 10964–10968.
Dunn, S. D. (1992). *J. Biol. Chem.* **267**, 7630–7636.
Eya, S., Maeda, M., and Futai, M. (1991). *Arch. Biochem. Biophys.* **284**, 71–77.
Fillingame, R. H., Jiang, W., and Dmitriev, O. Y. (2000). *J. Exp. Biol.* **203**, 9–17.
Garcia, J. J. and Capaldi, R. A. (1998). *J. Biol. Chem.* **273**, 15940–15945.
Gardner, J. L. and Cain, B. D. (1999). *Arch. Biochem. Biophys.* **361**, 302–308.
Gardner, J. L. and Cain, B. D. (2000). *Arch. Biochem. Biophys*, **380**, 201–207.
Girvin, M. E., Rastogi, V. K., Abildgaard, F., Markley, J. L., and Fillingame, R. H. (1998). *Biochemistry* **37**, 8817–8824.
Hartzog, P. E. (1994). Doctoral dissertation, University of Florida, Gainesvelle.
Hartzog, P. E. and Cain, B. D. (1993a). *J. Bacteriol.* **175**, 1337–1343.
Hartzog, P. E. and Cain, B. D. (1993b). *J. Biol. Chem.* **268**, 12250–12252.
Hartzog, P. E. and Cain, B. D. (1994). *J. Biol. Chem.* **269**, 32313–32317.
Hartzog, P. E., Gardner, J. L., and Cain, B. D. (1999). *Intern. J. Biochem. Cell Biol.* **31**, 769–776.
Hatch, L. P., Cox, G. B., and Howitt, S. M. (1998). *Biochim. Biophys. Acta.* **1363**, 217–223.
Jones, P. C. and Fillingame, R. H. (1998). *J. Biol. Chem.* **273**, 29701–29705.
Kato-Yamada, Y., Noji, H., Yasuda, R., Kinosita, K., Jr, and Yoshida, M. (1998). *J. Biol. Chem.* **273**, 19375–19377.
Kuo, P. H. and Nakamoto, R. K. (2000). *Biochem. J.* **347**, 797–805.
Lightowlers, R. N., Howitt, S. M., Hatch, L., Gibson, F., and Cox, G. B. (1987). *Biochim. Biophys. Acta.* **894**, 399–406.
McCormick, K. A. and Cain, B. D. (1991). *J. Bacteriol.* **173**, 7240–7248.
McCormick, K. A., Deckers-Hebestreit, G., Altendorf, K., and Cain, B. D. (1993). *J. Biol. Chem.* **268**, 24683–24691.
McLachlin, D. T., Coveny, A. M., Clark, S. M., and Dunn, S. D. (2000). *J. Biol. Chem.* **275**, 17571–17577.
Mendel-Hartvig, J. and Capaldi, R. A. (1991). *Biochemistry* **30**, 10987–10991.
Nakamoto, R. K., Ketchum, C. J., and Al-Shawi, M. K. (1999). *Annu. Rev. Biophys. Biomol. Struct.* **28**, 205–234.
Noji, H., Yasuda, R., Yoshida, M., and Kinosita, K., Jr. (1997). *Nature (London)* **368**, 299–302.
Ogilvie, I. and Capaldi, R. A. (1999). *FEBS Lett.* **453**, 179–182.
Rahman, S., Blok, R. B., Dahl, H. M., Danks, D. M., Kirby, D. M., Chow, C. W., Christodoulou, J., and Thorburn, D. R. (1996). *Ann. Neurol.* **39**, 343–351.
Rastogi, V. K. and Girvin, M. E. (1999). *Nature (London)* **402**, 263–268.
Revington, M., McLachlin, D. T., Shaw, G. S., and Dunn, S. D. (1999). *J. Biol. Chem.* **274**, 31094–31101.
Rodgers, A. J. W. and Capaldi, R. A. (1998). *J. Biol. Chem.* **273**, 29406–29410.
Sabbert, D., Engelbrecht, S., and Junge, W. (1997). *Proc. Natl. Acad. Sci. USA* **94**, 4401–4405.
Sambogni, Y., Iko, Y., Tanabe, M., Omote, H., Iwamoto-Kihara, A., Ueda, I., Yanagida, T., Wada, Y., and Futai, M. (1999). *Science*, **286**, 1722–1724.
Schemidt, R. A., Qu, J., Williams, J. R., and Brusilow, W. S. (1998). *J. Bacteriol.* **180**, 3205–3208.
Singh, S., Turina, P., Bustamante, C. J., Keller, D. J., and Capaldi, R. A. (1996). *FEBS Lett.* **397**, 30–34.
Sorgen, P. L., Bubb, M. R., McCormick, K. A., Edison, A. E., and Cain, B. D. (1998a). *Biochemistry* **37**, 923–932.
Sorgen, P. L., Caviston, T. L., Perry, R. C., and Cain, B. D. (1998b). *J. Biol. Chem.* **273**, 27873–27878.
Sorgen, P. L., Bubb, M. R., and Cain, B. D. (1999). *J. Biol. Chem.* **274**, 36261–36266.
Stock, D., Leslie, A. G. W., and Walker, J. E. (1999). *Science* **286**, 1700–1705.
Valiyaveetil, F. I. and Fillingame, R. H. (1997). *J. Biol. Chem.* **272**, 32635–32641.
Wada, T., Long, J. C., Zhang, D., and Vik, S. B. (1999). *J. Biol. Chem.* **274**, 17353–17357.
Wallace, D. C. (1999). *Science* **283**, 1482–1488.
Wilkens S. and Capaldi, R. A. (1998). *Nature (London)* **393**, 29.